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Hepatic Stellate Cells Functional Heterogeneity in Liver Cancer

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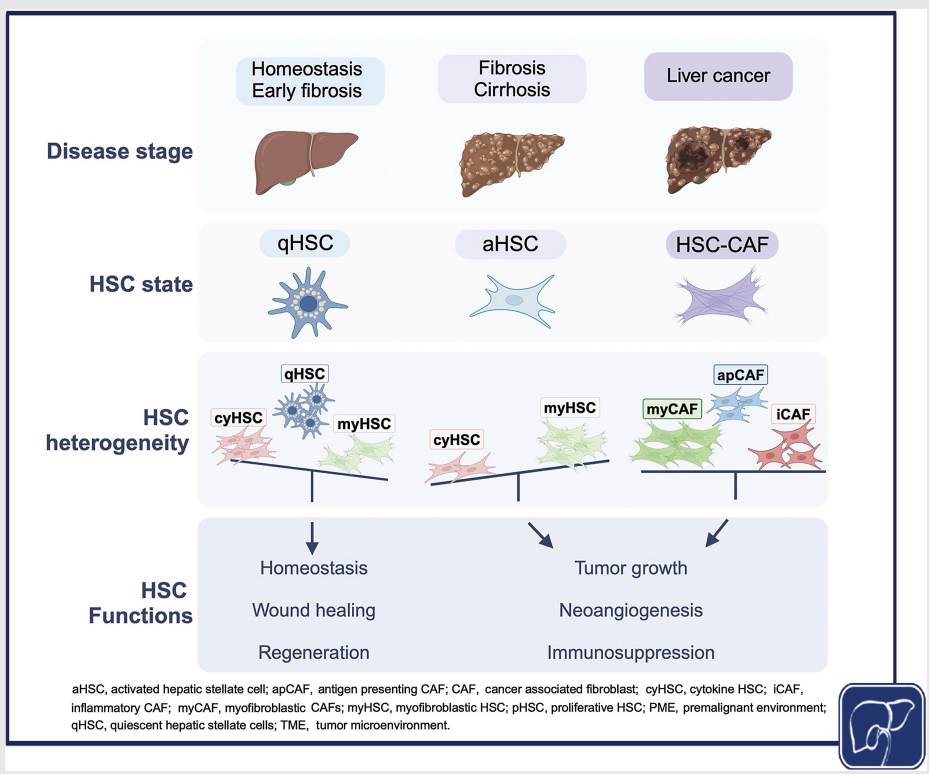
Graphical Abstract



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Abstract

Hepatic stellate cells (HSCs) are the liver's pericytes, and play key roles in liver homeostasis, regeneration, fibrosis, and cancer. Upon injury, HSCs activate and are the main origin of myofibroblasts and cancer-associated fibroblasts (CAFs) in liver fibrosis and cancer. Primary liver cancer has a grim prognosis, ranking as the third leading cause of cancer-related deaths worldwide, with hepatocellular carcinoma (HCC) being the predominant type, followed by intrahepatic cholangiocarcinoma (iCCA). Moreover, the liver hosts 35% of all metastatic lesions. The distinct spatial distribution and functional roles of HSCs across these malignancies represent a significant challenge for universal therapeutic strategies, requiring a nuanced and tailored understanding of their contributions. This review examines the heterogeneous roles of HSCs in liver cancer, focusing on their spatial localization, dynamic interactions within the tumor microenvironment (TME), and emerging therapeutic opportunities, including strategies to modulate their activity, and harness their potential as targets for antifibrotic and antitumor interventions.

Keywords

- hepatic stellate cells
- liver cancer
- cancer-associated fibroblasts
- tumor microenvironment

Lay Summary

With this review we highlight recent advances in understanding HSCs and their roles in liver cancer. We examine origins, localization, heterogeneity, and fate of HSCs, highlighting their contributions to the fibrotic premalignant environment and the tumor microenvironment in HCC and iCCA. HSCs and their mediators exert diverse effects, with some promoting cancer progression while others play protective roles. These findings inform the development of therapeutic strategies, encompassing CAR T cell technologies, to target specific HSC subtypes and functions, and combined treatments to improve current FDA-approved therapies.

Liver cancer was the sixth most diagnosed cancer, and the third leading cause of cancer-related mortality worldwide in 2020.[1] Hepatocellular carcinoma (HCC) accounts for 85% of all primary liver cancers, followed by intrahepatic cholangiocarcinoma (iCCA) which accounts for 15%.[2] The liver is also a frequent site for solid tumor metastases, such as colorectal cancer (CRC) and pancreatic cancer.[3,4] Liver cancer is often described as a "wound that does not heal," underscoring the complex interplay of inflammation, fibrosis, and tumor progression. Moreover, the pathways and environment driving HCC and iCCA differ. Indeed, HCC typically arises in a fibrotic liver, while fibrosis in iCCA develops concurrently within the tumor mass, suggesting distinct mechanisms of tumor initiation and growth. This review focuses on hepatic stellate cells (HSCs), the liver's resident pericytes from homeostasis to fibrosis and cancer. HSCs are key contributors to the fibrotic premalignant environment (PME) in which most HCC arise,[5] and to the HSC-derived cancer-associated fibroblast (CAF) pool that shapes the desmoplastic tumor microenvironment (TME) characteristic of iCCA. Fibrosis plays a central role in the pathogenesis of HCC, driven by conditions such as viral hepatitis, metabolic dysfunction-associated steatotic liver disease (MASLD), metabolic-associated steatohepatitis (MASH), smoke, alcohol, and obesity. These risk factors, compounded by heavy and binge drinking, have been shown to accelerate chronic liver injury, ultimately fostering fibrosis and a PME conducive to tumor development.[6–8] In contrast, iCCA is strongly associated with primary sclerosing cholangitis and liver fluke infections; however, it also shares overlapping risk factors with HCC including cirrhosis and metabolic dysfunction.[2] HSCs have been shown to be key players in both the PME and TME of primary and secondary liver malignancies.[9–11] While HSCs promote tumor initiation and progression by facilitating a fibrotic PME, where most HCCs arise, they contribute to the desmoplastic TME in iCCA, overall contributing to tumor growth.[12–14] However, due to their vital role in liver regeneration and homeostasis, HSC therapeutic targeting presents unique challenges that require careful consideration. This review explores the spatial localization, activation mechanisms, and functional heterogeneity of HSCs in the PME and TME of liver cancer, to highlight their contributions to tumor biology and evaluate their potential and implications as therapeutic targets in clinical settings, based on the current knowledge and future perspectives.

HSCs in Liver Homeostasis and Fibrosis

HSCs in Liver Homeostasis

HSCs are the largest population of resident mesenchymal cells in the liver and are localized in the space of Disse, between the hepatocytes and the liver sinusoidal endothelial cells (LSEC). They account for approximately 4 to 10% of all liver cells[15] and proliferate dramatically during liver fibrosis. Quiescent HSCs (qHSC) are cells of mesodermal origin,[16] and can be recognized by the expression of several markers including but not limited to *LRAT*, *DES*, *RGS5*, *RELN*, *COLEC11*, and *GFAP*.[17–19] During liver morphogenesis, the mesodermal septum transversum mesenchyme gives rise to mesothelial cells, which in turn

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differentiate into both HSCs and perivascular mesenchymal cells, including other liver fibroblast populations such as portal fibroblasts and vascular smooth muscle cells.[20]

Transcriptomic profiling and staining of healthy mouse liver revealed HSC zonation, with portal vein-associated HSCs (PaHSCs) expressing *Ngfr* and *Itgb*, and central vein-associated HSCs (CaHSCs) expressing *Adamtsl2* and *Rspo3*.[21] Such zonation has also been confirmed in human livers through the expression of NGFR and ADAMTSL2, along with the identification of GPC3+ HSCs, localized around the portal and central regions, and DBH^+ HSCs predominantly found in the perisinusoidal space,[22] suggesting a link between spatial and functional specialization, which requires further exploration.

Although activated HSCs (aHSCs) have been extensively investigated, the function of qHSCs remains elusive in comparison, partly due to limitations in experimental models. Indeed, the spontaneous activation of HSCs *in vitro* makes it challenging to investigate their role in a quiescent state. Through the enzyme lecithin retinol acyltransferase (LRAT), qHSCs are the primary storage site for vitamin A in the body, accounting for 80 to 90% of the total vitamin A.[23] Beyond this metabolic role, in homeostasis, qHSCs also contribute to maintaining the turnover of the extracellular matrix (ECM) by regulating sinusoidal blood flow through vasoconstriction, and releasing growth factors critical for liver homeostasis.[9,24] With their long "star-like" shape and rich ligand–receptor repertoire, HSCs exhibit extensive interactions with parenchymal and non-parenchymal cells.[13,25] Direct cell–cell contact with hepatocytes and LSECs enables both paracrine and contact-dependent signaling,[24] with LSECs playing a pivotal role in maintaining HSC quiescence through physical proximity and sinusoidal signaling.[26]

HSC-depletion studies using gliotoxin and glial fibrillary acidic protein (GFAP)-thymidine kinase strategies[27] have suggested divergent roles for HSCs in liver regeneration. More recent and refined approaches, using "Jedi" T cells engineered to deplete GFP-expressing $PDGFR\beta^+$ (platelet derived growth factor receptor beta) cells,[28] have shown a critical role for qHSCs in maintaining liver zonation and mass, mediated by the release of neurotrophin-3 (NTF3), which promotes hepatocyte proliferation and liver regeneration.[28] However, although the homeostatic functions of qHSCs are becoming clearer, further studies are needed to unravel the full scope of their dynamic interactions, signaling, and broader impact on liver physiology.

Myofibroblasts Origin and Heterogeneity

Upon liver injury, qHSCs transdifferentiate into aHSCs, fibrogenic myofibroblasts, acquiring proliferating and migrating capacities, allowing them to migrate to the site of the wound.[29] This activation process is regulated by multiple signaling molecules and pathways, with transforming growth factor-β (TGF-β) playing a central role in driving activation and platelet-derived growth factor (PDGF) significantly contributing to HSC proliferation.[29] Transcriptomic analyses suggest that this activation program is conserved across various liver injury etiologies, including toxic, biliary, and fatty liver diseases, reflecting a shared core activation program.[13,19] During activation, HSCs lose their lipid droplets and retinoid-storing capacity, transitioning into a fibrogenic state characterized by excessive ECM production, leading to tissue stiffening, vasoconstriction, and loss of sinusoidal fenestration. Although this process is key for tissue repair and wound healing, chronic HSC activation exacerbates liver fibrosis, impairs liver function, and creates a favorable "soil" for HCC emergence.[13] Lineage-tracing studies have identified HSCs as the predominant source of myofibroblasts in liver fibrosis across multiple etiologies,[18,24,30,31] followed by portal fibroblasts, which play a larger role in cholestatic liver diseases.[32] The contribution of vascular smooth muscle cells (VSMCs),[33] mesothelial cells,[20] and bone-marrow derived fibroblasts[34] to the HSC pool appears minimal or absent based on the published literature, and remains poorly investigated (►**Fig. 1**).

The characterization of HSC heterogeneity can be influenced by both computational factors, such as clustering methodologies, and biological factors, including the stages of HSC activation[19,35] as well as their spatial localization.[36] These variations complicate the establishment of a universal nomenclature and underscore the spatial and dynamic roles of HSCs as they respond to and participate in different stages of liver injury and fibrosis progression. Previous reviews have proposed classifications for HSC subtypes in mouse models.[14,37] Besides qHSCs, HSCs in fibrotic conditions can be broadly categorized into four main groups, cytokine and growth factor enriched (cyHSCs), myofibroblastic (myHSCs), proliferating, and senescent, each associated with specific functions, as summarized in ►**Table 1**.

However, a key limitation in understanding the role of these HSC subtypes is the lack of tools to isolate them and maintain their transcriptional phenotype *in vitro*, as well as the difficulty of manipulating them *in vivo*, therefore reducing our ability to examine their roles in specific states, and complicating efforts to target them selectively without impacting the entire HSC population.[13]

Fate of HSCs After Activation

After activation, HSCs can follow three main fates: apoptosis, inactivation, and senescence[38,39] (►**Fig. 1**). Based on the recent knowledge describing the presence of transcriptionally heterogeneous HSCs, it remains unclear if all HSCs harbor these capabilities. After the resolution of liver damage, around half of aHSCs are estimated to undergo apoptosis, while the other half become inactivated (iHSCs). Although iHSCs initially display a phenotype similar to qHSCs, with reduced expression of fibrogenic genes and increased expression of genes related to the quiescent state, iHSCs exhibit a higher capacity for reactivation compared to qHSCs.[39–42] Additionally, aHSCs can undergo senescence (SenHSCs), a state characterized by stable growth arrest, accompanied by the release of a pro-inflammatory secretome known as the senescence-associated secretory phenotype (SASP).[42–44] SenHSCs are estimated to constitute 2 to 8% of the HSC pool, and despite showing increased ECM production compared to qHSC, they produce less ECM than aHSCs.[45] Moreover, SenHSCs are enriched in ECM-degrading enzymes, suggesting a potential role in promoting fibrosis resolution.[42] However, SenHSCs can also contribute to a

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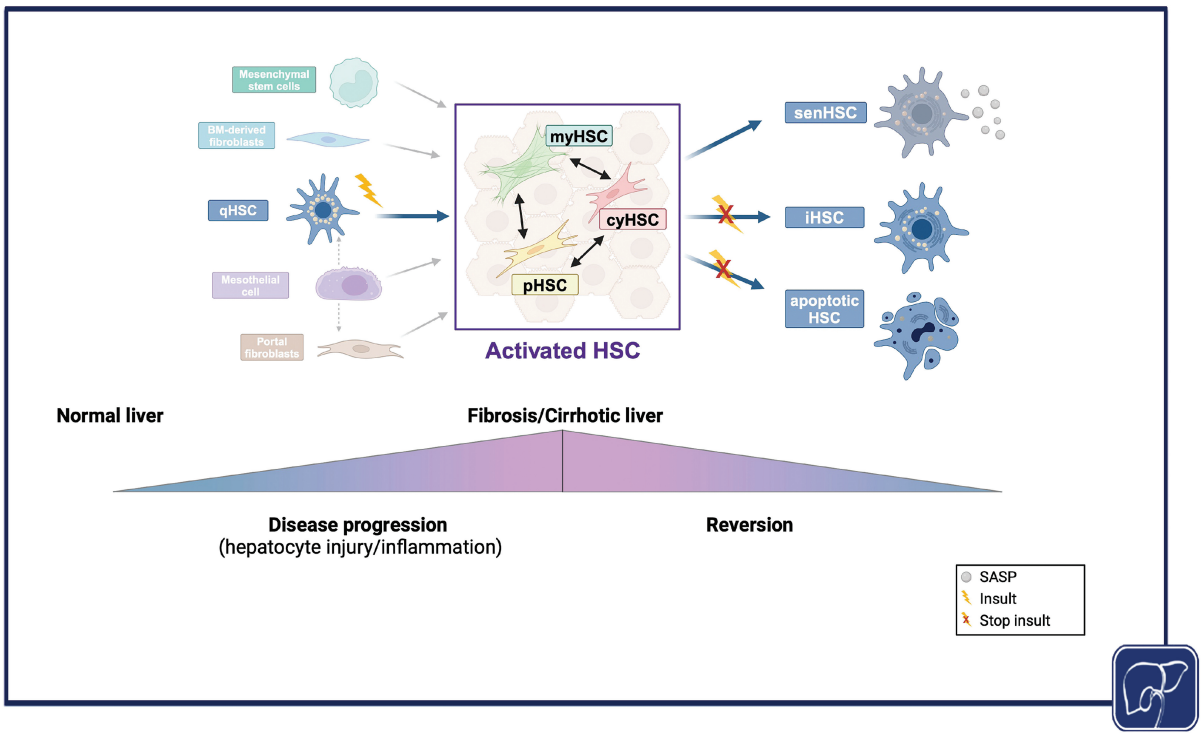

Fig. 1 Trajectories of hepatic stellate cell (HSC): from homeostasis to activation and resolution. Quiescent hepatic stellate cells (qHSCs) are the primary source of myofibroblasts in liver fibrosis. Additional cell types also slightly contribute to the myofibroblast pool (gray arrows). Upon liver injury, qHSCs transdifferentiate into heterogeneous activated HSC (aHSC) subtypes: cytokine/growth factor-enriched HSCs (cyHSCs), myofibroblastic HSCs (myHSCs), and proliferative HSCs (pHSCs). After activation, HSCs can undergo senescence, characterized by growth arrest and a senescence-associated secretory phenotype (SASP), which can modulate the liver environment and influence disease progression. Upon injury removal, during fibrosis resolution, HSCs may undergo apoptosis or reversion toward an inactivated state (iHSC). cyHSC, cytokine HSC; iHSC, inactivated HSC; myHSC, myofibroblastic HSC; pHSC, proliferative HSC; qHSC, quiescent HSC; SASP, senescence-associated secretory phenotype; senHSC, senescent HSC.

Table 1 HSC subtypes in the fibrotic PME

HSC subtype	Markers	Functions	References
Cytokine and growth factor enriched HSC (cyHSC)	Lowly activated HSC or *Col1a1*^{low}, enriched in growth factors (e.g., *Rspo3*, *Hgf*) and cytokines (e.g., *Cxcl12*)	Support hepatocyte survival and regeneration through growth factors and cytokines	Filliol et al, 2022[13]
Myofibroblastic HSC (myHSC)	Highly activated HSC *Col1a1*^{high} express activation markers (e.g., *Acta2*) and ECM proteins (e.g., *Col1a1*)	Remodel ECM, increase liver stiffness, and promote pro-tumorigenic changes in advanced fibrosis	Filliol et al, 2022[13] Kostallari et al, 2022[195] Krenkel et al, 2019[196]
Proliferative HSC	*Ki67*+ *Top2a*+ Actively dividing HSCs contributing to expansion of HSC population	Expand HSC populations and support fibrotic responses	Merens et al, 2024[19]
Senescent HSC	Cell cycle arrest genes (*Cdkn1a*/p21, *Cdkn2a*/p16), SA-βGal+, SASP markers, uPAR	Promote chronic inflammation, alter ECM remodeling, and contribute to fibrosis persistence	Krizhanovsky et al, 2008[42] Takahashi et al, 2018[43] Burton et al, 2014[44] Yashaswini et al, 2024[45] Yoshimoto et al, 2013[46] Lujambio et al, 2013[47] Amor et al, 2020[48] Li et al, 2020[49]

Abbreviations: ECM, extracellular matrix; HSC, hepatic stellate cell; PME, premalignant environment.

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detrimental inflammatory environment, damaging surrounding cells and tissue.[42]

However, the role of SenHSCs remains controversial.[13,46–48] While the deletion of key senescence regulators in HSCs has been shown to exacerbate HSC activation and fibrosis,[47] therapeutic strategies targeting SenHSCs, such as senolytic therapies or chimeric antigen receptor (CAR) T cells directed against uPAR+ (urokinase-type plasminogen activator receptor) cells, have demonstrated efficacy in reducing HCC progression.[48,49] Indeed, while uPAR is upregulated by senescent cells, it can also be expressed by Trem2+ macrophages, and their targeting may contribute to the antifibrotic effects of CAR T cells.[45] Although no universal marker for senescence exists, these findings highlight the potential of targeting SenHSCs as a novel therapeutic approach for liver fibrosis and TME modulation in liver cancer.

Functional Roles of HSCs in HCC

Chronic liver disease creates a specific PME, a fibrotic niche that precedes the onset of HCC. This PME is characterized by chronic hepatic cell death, inflammation, and fibrosis.[50] Genetic studies utilizing LratCre-based models have demonstrated an overall pro-tumoral role for HSCs during HCC development.[13,51] However, HSCs can also exhibit antitumoral properties under specific conditions, reflecting their complex and context-dependent functions, which will be discussed in this section.

Histological Fibrotic Features

In most HCC patients, HSCs predominantly accumulate in non-malignant fibrotic regions of the liver, such as the fibrotic septa, around hepatic blood sinusoids, and within the tumor capsule, contributing to a stiff adjacent liver but a relatively soft HCC TME[52] (►**Fig. 2**). This fibrotic architecture is central to the PME, where HSCs influence hepatocyte survival, proliferation, and the early stages of malignant transformation. Indeed, analysis of HSC signatures and more specifically aHSC signatures in the PME can predict HCC risk and survival.[13,53]

However, some HCC subtypes exhibit a fibrotic TME rather than the typical soft tumor core. Rare variants, such as scirrhous HCC and fibrolamellar HCC, are characterized by a dense fibrotic stroma.[52] Similarly, steatohepatitic HCC (SH-HCC) in hepatitis C virus–related cirrhosis with associated MAFLD and MASH has also been described to display interstitial fibrosis, though to a lesser extent.[54] Importantly, CAF density within the HCC lesion has been correlated with poor survival.[55,56] Therefore, aHSCs in the PME as well as CAFs in the TME both seem to exert an overall tumor-promoting roles in HCC.

The ECM, also referred to as the matrisome, is a dynamic and ever-evolving network consisting of over 300 macromolecules, including collagens, proteoglycans, and glycoproteins.[57,58] These molecules provide structural support to cells and tissues while serving as a reservoir for growth factors and cytokines that regulate cellular behavior. Although several

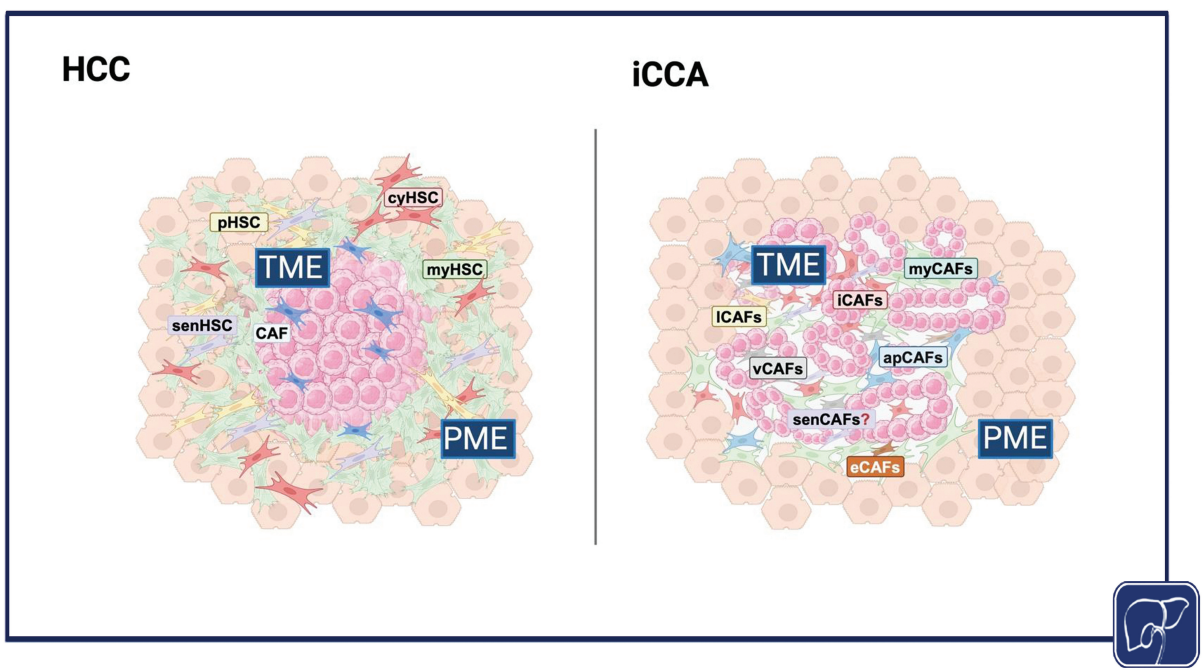

Fig. 2 Spatial differences in fibroblasts location between hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA). Activated hepatic stellate cells (HSCs) accumulate in the premalignant environment (PME) in most HCC cases, with limited infiltration into the tumor microenvironment (TME). CyHSCs, myHSCs, pHSCs, and SenHSCs subtypes contribute to the pro-fibrotic PME milieu, by directly and indirectly supporting tumor initiation and progression. In iCCA HSC-derived cancer-associated fibroblasts (CAFs) are abundant and accumulate within the tumor mass in the TME, where myCAFs are the most abundant subtype followed by iCAFs. Other CAF subtypes, such as apCAf, vCAFs, eCAFs, lCAFs, and eventually SenCAFs, have been also described in the TME of iCCA. apCAF, antigen presenting CAF; CAF, cancer associated fibroblast; cyHSC, cytokine HSC; eCAF, EMT-like CAF; iCAF, inflammatory CAF; lCAF, lipo-fibroblasts; mesCAF, mesothelial CAF; myCAF, myofibroblastic CAFs; myHSC, myofibroblastic HSC; pHSC, proliferative HSC; PME, premalignant environment; SenCAF, senescent CAF; TME, tumor microenvironment; vCAF, vascular CAF.

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ECM components have been linked to tumor progression, only a few have been studied *in vivo* through perturbation experiments, highlighting the need for further research. Among these components, collagen type I is the most abundant fibrillar collagen in the PME. Although it can provide mechanical stability, its excessive accumulation increases liver stiffness, a hallmark of advanced fibrosis.[58] Indeed, liver stiffness values exceeding 12 kPa have been strongly correlated with an elevated risk of HCC.[59–61] Furthermore, persistent stiffness, even after HCV eradication, is associated with continued risk for decompensated cirrhosis and HCC.[62] Beyond its mechanical properties, the ECM acts as a reservoir for growth factors such as TGF-β and other cytokines, released during matrix remodeling, to promote HSC activation and hepatocyte proliferation.[63]

In the PME of HCC, regenerative nodules—and later dysplastic nodules, composed of proliferating hepatocytes—frequently arise encapsulated by fibrotic septa. As these nodules expand, fibrosis remains mainly external rather than infiltrating the nodule itself, which may contribute to the softer structure observed in HCC compared to the surrounding fibrotic PME. Dysplastic nodules, which exhibit atypical hepatocyte morphology and are frequently associated with mutations in oncogenic pathways linked to HCC,[64,65] are frequently found in cirrhotic settings, suggesting the potential contribution of HSCs to malignant transformation. Although the transition from cirrhosis to dysplastic nodules remains poorly understood, HSC may contribute to this process by promoting hepatocyte survival, remodeling the ECM, and modulating the immune microenvironment to favor malignant transformation.

HSCs as Dual Mediators of Hepatocyte Survival and HCC Progression

As in the healthy liver, HSCs also interact with hepatocytes in the PME, and contribute to their proliferation and survival.[13] In mouse models, collagen type I, predominantly expressed by myHSCs, promotes HCC development in fibrotic livers, where it contributes to stiffness.[13] However, this effect is absent in milder fibrosis with lower stiffness,[13] aligning with clinical observations that liver stiffness correlates with increased HCC risk in humans.[59–61] Mechanistically, collagen-induced stiffness activates mechanosensitive and oncogenic pathways, such as transcriptional coactivator with PDZ-binding motif (TAZ) in hepatocytes.[13] TAZ partners with TEA domain transcription factor 2 (TEAD2) and TEAD4, which drive proliferation of malignant hepatocytes and increase their programmed cell death ligand 1 (PD-L1) expression,[66] thereby promoting tumor initiation and progression[13,66] while fostering an immunosuppressive environment (►**Fig. 3**). Other HSC-derived pro-tumoral factors affecting hepatocytes transformation have already been largely discussed.[5,66,67]

Conversely, fibroblasts have also been shown to exert anti-tumor effects. This concept, known as "neighbor suppression," was earlier described after observing that normal

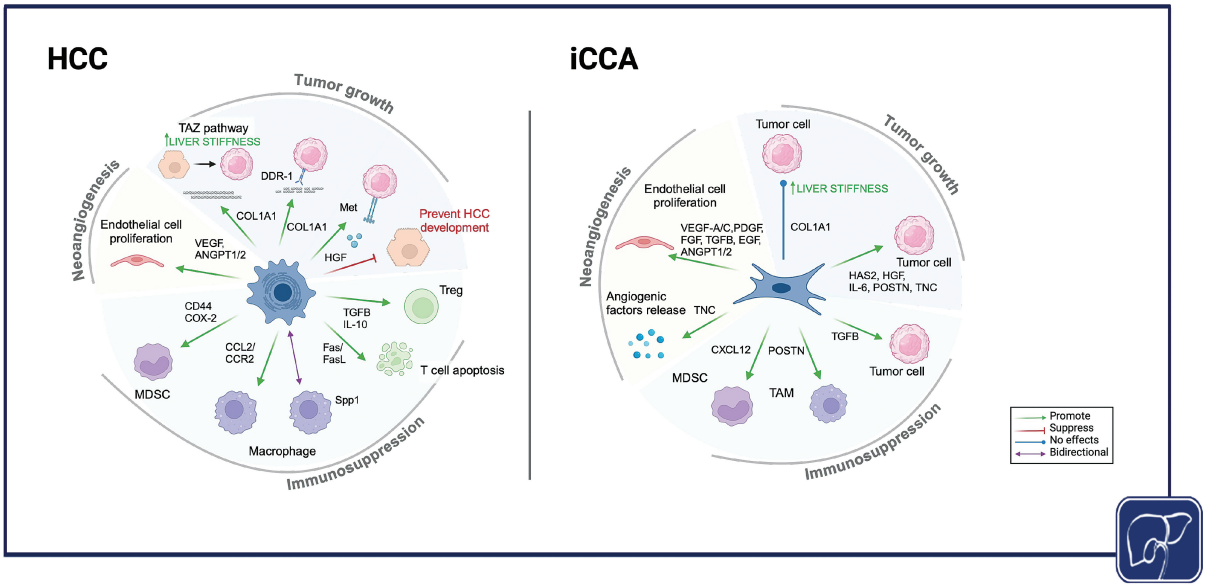

Fig. 3 Functions and mediators of activated hepatic stellate cell (aHSC) in hepatocellular carcinoma (HCC) and HSC-derived cancer-associated fibroblast (CAF) in intrahepatic cholangiocarcinoma (iCCA). aHSC in HCC and HSC-derived CAFs in iCCA produce a wide range of mediators that can modulate critical pathways affecting tumor growth, neoangiogenesis, and immunosuppression, among others. Depending on the context, those mediators can be tumor promoting, tumor restrictive, or have no effects on the premalignant environment (PME) nor the tumor microenvironment (TME). Here are shown few of these mediators. ANGPT1/2, angiopoietin; CCL2, C-C motif chemokine ligand 2; CCR2, C-C motif chemokine receptor 2; CD44, cluster of differentiation 44; COL1A1, collagen type 1 alpha 1 chain; COX-2, cyclooxygenase-2; CXCL12, C-X-C motif chemokine ligand 12; DDR-1, discoidin domain receptor 1; EGF, epidermal growth factor; Fas, Fas cell surface death receptor; FasL, Fas ligand; FGF, fibroblast growth factor; HAS2, hyaluronan synthase 2; HGF, hepatocyte growth factor; IL-6, interleukin 6; IL-10, interleukin 10; MDSC, myeloid-derived suppressor cell; MET, MET proto-oncogene receptor tyrosine kinase; PDGF, platelet derived growth factor; POSTN, periostin; Spp1, secreted phosphoprotein 1; TAM, tumor associated macrophage; TGF-β, transforming growth factor beta; TNC, tenascin; Treg, regulatory T cells; VEGF, vascular endothelial growth factor.

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fibroblasts can inhibit the growth of transformed cells.[68–70] In the healthy and fibrotic liver, hepatocyte growth factor (HGF), mainly secreted by cyHSCs, has been recently shown to protect hepatocytes by activating pro-survival signaling pathways.[13] However, as cyHSCs abundance declines in advanced fibrosis, this protective effect seems to diminish during tumorigenesis. Similarly, we can speculate that iHSCs, being less activated, might also confer this supportive role. Although "neighbor suppression" may also involve contact-dependent mechanisms, further investigation is needed to fully understand the range of protective factors involved. These findings highlight the dual roles of HSCs, with their effects dependent on localization and fibrosis severity.

HSC Interactions with Non-parenchymal Cells During HCC Progression

HSCs engage in diverse interactions with immune, endothelial, and self-subpopulations playing a critical role in HCC progression. In the PME, HSCs have been shown to secrete chemokines and cytokines, sustaining a pro-tumorigenic and immunosuppressive environment (►**Fig. 3**). Indeed, HSCs have been shown to promote regulatory T cell (Treg) differentiation and survival,[71–75] suppress cytotoxic T cell activity,[74–80] and impair NK cell function.[81–84] Additionally, HSCs facilitate the conversion of monocytes into myeloid-derived suppressor cells (MDSCs), while skewing macrophages toward an anti-inflammatory and immunosuppressive phenotype.[51,53,85] They also inhibit dendritic cell (DC) maturation, and induce immunosuppressive PD-L1+ neutrophils (►**Fig. 3**). The mechanisms by which HSCs affect immune cells are summarized in ►**Table 2**.

These bidirectional interactions further support HSC activation and survival. Indeed, pro-inflammatory cytokines mainly produced by macrophages, such as tumor necrosis factor alpha (TNF-α), IL-1β, and IL-6, promote HSC survival,[86] while TGF-β and PDGF stimulate HSC activation and proliferation.[29] Furthermore, Tregs enhance HSC activation and contribute to liver fibrosis *via* secretion of IL-8 and amphiregulin.[72,87] TGF-β is a central mediator in HSC–immune cells crosstalk, bridging fibrosis and immune evasion in liver cancers. TGF-β is produced by various cell types in the PME and TME, including myofibroblasts, T cells, macrophages, tumor cells, and endothelial cells, and is also stored within the ECM. Although TGF-β has antiproliferative effects on epithelial cells, tumor cells in advanced HCC often develop insensitivity to it, shifting its role to a predominantly tumor-promoting function by inducting EMT features in HCC cells.[88] TGF-β's immunosuppressive functions include, but are not limited to, N1-to-N2 neutrophil polarization and enhancement of Treg differentiation, while inhibiting cytotoxic T cells and NK cells activity. Importantly, TGF-β inhibition has shown promise in enhancing antitumor responses, particularly in combination with immune checkpoint inhibitors, highlighting its critical role in the immunosuppressive TME.[89]

HSCs and LSECs exhibit a dynamic interplay that influences each other's behavior and plays a critical role in liver disease progression and tumor development. The loss of the unique fenestrated surface displayed by LSECs has been described as an early event that precedes fibrosis across various etiologies.[90] In the healthy liver, LSECs help maintain HSCs in their quiescent state, contributing to tissue homeostasis.[90,91] However, in pathological conditions, such as increased liver stiffness, *in vitro* studies have suggested that LSECs can promote HSC activation, further driving fibrosis and altering the hepatic microenvironment.[92] Activated HSCs in turn secrete proangiogenic factors, such as vascular endothelial growth factor (VEGF) and angiopoietins (Angpt1 and Angpt2), which enhance endothelial cell proliferation, increase vessel permeability, and promote neovascularization ultimately fueling tumor growth.[93,94] The importance of targeting HSC–LSEC interactions is also underscored by a study showing that inhibition of the Angpt2/Tie2 signaling pathway as a strategy to block angiogenesis not only reduces fibrosis but also attenuates MASH-induced HCC development.[95] Additionally, anti-angiogenic therapies (e.g., VEGF targeting) have become a cornerstone in the treatment of advanced HCC, particularly in combination with immune checkpoint blockade (ICB) therapies, demonstrating improved survival outcomes in patients with advanced HCC.[96–98]

Finally, computational cell–cell interaction algorithms have predicted paracrine and autocrine communication between different HSC subpopulations themselves in the fibrotic liver and TME of ICC.[13,25,99] Although these predictions remain speculative and require *in vivo* validation, a recent study uncovered an HSC autocrine circuit where NTF3–NTRK3 interaction promoted liver fibrosis in MASH.[25]

Role of HSCs in the TME of HCC

Although most HCCs exhibit low intratumoral fibrosis, multiple studies have identified a subset (approximately 10%) with an active stroma aligning with the S1 subclass of the Hoshida classification.[100] These HCCs, often characterized by "fibrous nests," display high WNT/TGF-β signaling activity, a stemness profile or an onco-fetal signature, T cell exhaustion, and poorer prognosis compared to other HCC subtypes.[100–103] Recent spatial immune profiling studies revealed that SP-HI (highly immune-infiltrated) HCC subtype, enriched in lymphocytes—particularly B cells and CD8+ T cells—and stroma infiltration, may exhibit increased sensitivity to immune checkpoint blockade (ICB) therapy.[104–106] Conversely, the SP-PF (proliferative) HCC subtype, marked by elevated AFP and sparse infiltration of CD8+ T cells and B cells, exhibits an "immune-excluded" environment where CD8+ T cells are largely restricted from the tumor core. SP-PF displays extensive fibroblast–immune cell interactions with Tregs, macrophages, and CD4+ T cells, creating a highly immune-suppressive environment that may limit ICB responsiveness.[104] Indeed, prior research showed that a tumor immune barrier formed by CAFs and immunosuppressive cells like SPP1+ macrophages may act as a physical blockade to immune cell infiltration, contributing to anti-programmed cell death 1 (anti-PD-1) treatment resistance.[107,108] Interestingly, in HCC patients responding to neoadjuvant cabozantinib and nivolumab therapy, CAFs with pro-inflammatory signaling were associated

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Table 2 HSC–immune cells interaction contributing to the immunosuppressive and pro-tumorigenic environment in HCC

Cell types	Mechanisms	Effects	References
Tregs	Promote Treg differentiation and survival; Treg reduces NK-mediated clearance of HSCs Enhance Treg immunosuppressive activity via TGF-β and IL-10	Immunosuppression and persistence of activated HSCs	Chou et al, 2011[71] Langhans et al, 2013[72] Dunham et al, 2013[73] Zhao et al, 2012[74] Dangi et al, 2012[75]
Cytotoxic T cells	Induce T cell apoptosis via Fas/FasL; suppress PD-L1 signaling, inhibit proliferation, and reduce cytotoxicity	Reduced cytotoxicity, leading to immune evasion	Zhao et al, 2012[74] Charles et al, 2013[76] Yu et al, 2004[77] Xia et al, 2011[78] Xia et al, 2013[79] Dangi et al, 2012[75] Zhao et al, 2011[80]
Monocyte macrophages	Promote anti-inflammatory and immunosuppressive CD163+ macrophages via CCL2/CCR2 signaling aHSC attract Cx3cr1 + Arg1 + macrophages via Cx3cl1 in the peritumoral area	Enhanced production of anti-inflammatory cytokines; reduced pro-inflammatory responses Promote CD8+ T-cell suppression	Xi et al, 2021[85] Ji et al, 2015[53] Otto et al, 2023[51] Jeong et al, 2024[197]
MDSCs	Promote MDSC conversion via CD44 and COX-2 secretion Depend on IFNγ signaling in HSCs Increase HCC migration and tumor sphere formation	Increased immunosuppression and inflammation Promote HCC tumorigenesis	Höchst et al, 2013[198] Xu et al, 2016[199] Chou et al, 2011[71] Ji et al, 2015[53] Zhao et al, 2012[74] Dangi et al, 2012.[75] Seki et al, 2015[200]
DCs	Inhibit DC maturation and cytokine production; upregulate DIgR2 expression	Impaired antigen presentation; reduced Th1 responses	Flavell et al, 2010[201]
NK cells	Inhibit NK cell activity via TGF-β, PGE2, and ECM-associated mechanisms	Immune escape via reduction for tumor cell killing	Li et al, 2012[81] Shi et al, 2017[82] Katz et al, 2008[83] Becker et al, 2013[84]
Neutrophils	Induce PD-L1 expression on neutrophils through CAF-secreted mediators	Suppression of immune responses	Cheng et al, 2018[202]

Abbreviations: DC, dendritic cell; ECM, extracellular matrix; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; MDSC, myeloid-derived suppressor cell; Tregs, regulatory T-cells.

with enhanced responsiveness to immunosuppression.[109] However, one case of recurrence revealed interactions between CAFs and stemness-expressing HCC tumor cells, underscoring the complexity of stromal-tumor dynamics.[109] These findings align with a pan-cancer study that identifies both immune-excluded and immune-exhausted TME, with the immune-excluded setting—characterized by stromal barriers and TGF-β signaling—being particularly resistant to ICB.[110] Interestingly, SH-HCC, which accounts for approximately 23% of nonviral HCC cases,[111] is associated with higher levels of T cell exhaustion and stromal signatures, alongside TGF-β signaling activation. In a small cohort of 7 SH-HCC cases among 30 HCC patients, SH-HCC showed significantly longer progression-free survival (PFS) in response to atezolizumab plus bevacizumab therapy compared to non-steatotic HCC cases.[111]

The deposition of ECM activates multiple receptors, including discoidin domain receptor 1 (DDR1), a collagen receptor with pro-tumoral effects in breast cancer, HCC, and pancreatic ductal adenocarcinoma (PDAC). DDR1 binds to cleaved collagen, and promotes fiber alignment, facilitating immune evasion in triple-negative breast cancer.[112] In HCC, DDR1 is upregulated in fibrotic tissues and tumors, with hepatocyte-specific DDR1 knockout reducing proliferation in malignant, but not in non-malignant, hepatocytes.[13] Interestingly, transgenic mice expressing a non-cleavable form of collagen type I showed reduced HCC development, suggesting its potential mechanical restriction role.[113] Collagen cleavage, mediated by metalloproteinase (MMPs), is required for DDR1 binding, and high MMP expression correlates with poor outcomes in HCC.[13] Indeed, Col1-DDR1 signaling has also been associated with HCC stemness.[114] Similarly, the collagenolysis-DDR1 pathway has been associated with poor prognosis in PDAC.[115] Unlike the Col1-stiffness-TAZ pathway, which modifies the PME, the Col1-DDR1 pathway appears to drive tumor progression in established HCC experimental models[13] (►**Fig. 3**).

Molecularly, numerous CAF-derived molecules have been implicated in promoting HCC growth, as extensively reviewed elsewhere,[67,116] and some examples are illustrated in ►**Fig. 3**. However, much of our current understanding comes from *in vitro* experiments, which may not fully

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capture the complexity of CAF heterogeneity or their relevance to specific HCC subtypes. The recent identification of distinct CAF subpopulations highlights the need for *in vivo* validation to better understand their functional roles and therapeutic potential in HCC progression.

Functional Role of HSC-derived CAFs in the TME of iCCA

Lineage tracing and single cell RNA sequencing (scRNA-seq) revealed that HSCs are the primary source of CAFs not only in HCC, but also in liver metastasis and iCCA,[13,117–119] followed by other mesenchymal cells such as portal fibroblasts (PFs).[14,120] Unlike in HCC, where HSCs primarily reside in the PME, HSC-derived CAFs in iCCA coevolve with tumor cells, being an active part of the TME. This is not a unique feature of iCCA, but it is also shared with other tumors including PDAC, CRC, as well as their metastasis in the liver. CAFs are responsible for the exacerbated ECM production, leading to the dense desmoplastic stroma characteristic of these tumors, which increases stiffness and promotes poor prognosis and resistance to therapy.[121–124] Due to their contribution to pro-tumorigenic biological processes such as invasion and metastasis, angiogenesis, ECM modelling, and immunosuppression, CAFs have largely been associated with a tumor-promoting role in various tumors, including liver cancer.[99,124] Recent studies using transgenic mouse models that specifically deplete HSC-derived CAFs and αSMA^+ (alpha smooth muscle actine) CAFs, have demonstrated an overall tumor-promoting function of CAFs in iCCA and in liver metastasis models of PDAC and CRC.[117,118] Similarly, treatment with BH3-mimetic Navitoclax, used to kill CAFs in iCCA, reduced tumor progression.[125] Moreover, since Navitoclax can target senescent cells the potential of senescent CAF targeting in iCCA seems promising. However, this remains a poorly investigated area, with a study describing the association of senescent CAFs with lower tumor-infiltrating lymphocytes and higher PD-L1 expression in iCCA.[126]

In contrast to iCCA, αSMA^+CAFs in PDAC experimental models have been described as tumor restrictive, suggesting that the organ context, cell of origin, and subtype of CAFs are crucial considerations when developing targeted therapies.[127] This raises the question whether functionally diverse CAF populations exist in liver tumors as well. Indeed, these findings would underscore the presence of CAF functional heterogeneity and may open to new opportunity for therapy.

Histological Features of iCCA

Histologically, iCCA can be classified into large duct iCCA, originating from intrahepatic large bile ducts and often associated with pre-cancerous or pre-invasive lesions, and small duct iCCA, arising from intrahepatic small bile ducts, and linked to better survival outcomes.[128–130] In liver metastases, tumors can be categorized by distinct histopathological growth patterns (HGPs). Easily assessed by hematoxylin and eosin staining, these patterns are based on CAFs distribution and features at the tumor–liver interface, which have been associated with patient prognosis, and can predict response to therapy.[122] Although HGPs in iCCA remain largely unexplored, significant research has focused on liver metastases,[131,132] classifying them into two main types, (i) encapsulated, where a stromal capsule separates the tumor cells from the liver parenchyma; and (ii) non-encapsulated, where tumor cells are in direct contact with hepatocytes, which is associated with worse prognosis and an immunosuppressive environment.[122,133–136] Therefore, further investigation of HGPs in iCCA could enhance our understanding of tumor biology, aiding in patient stratification and in the development of new therapeutic strategies.

Heterogeneity of HSC-Derived CAFs

Building on scRNA-seq findings, the transcriptional heterogeneity of CAFs has become increasingly evident, with several studies identifying multiple CAF subtypes based on their transcriptomic profiles.[117,118,137,138] However, there is no universal consensus on the exact number nor nomenclature for these CAF subtypes. Indeed, depending on the resolution used in data analysis, the number of identified CAF subtypes varies, highlighting how CAF transcriptomic heterogeneity is often based on subjective criteria, rather than its objective functionality. Investigating CAF functionality should therefore be prioritized to better define CAF therapeutic potential. Cross-species studies in iCCA and liver metastasis consistently identified five main CAF subtypes related to their functional role: (1) myofibroblastic CAFs (myCAFs), enriched in ECM transcripts and involved in ECM remodeling; (2) inflammatory CAFs (iCAFs), enriched in cytokines, growth factors, and in genes regulating the inflammatory response; (3) antigen-presenting CAFs (apCAFs), expressing major histocompatibility complex II (MHC-II) and immunoregulatory genes; (4) vascular CAFs (vCAFs), enriched in genes for muscle contraction and vascular development and featuring vascular development-related transcripts; (5) EMT-like CAFs (eCAFs) expressing epithelial to mesenchymal markers (►**Fig. 2**). Other CAF populations, such as the lipo-fibroblasts (lCAFs), related to lipid metabolism, and the mesothelial CAF (mesCAF) expressing mesothelial markers, as well as senescent CAF (SenCAFs), have also been described, but require further validation.[117,118,124,126,137,138] Although transcriptional heterogeneity among CAFs holds potential for patient stratification, its utility in developing novel therapeutic strategies depends on understanding the functional role of specific CAF mediators rather than only pertaining to specific signatures and subtypes. This indeed remains challenging as previously discussed for HCC due to both technical and biological limitations. Moreover, the presence of hybrid iCAF/myCAF subtypes suggest CAF plasticity, which requires further investigation to better understand how CAFs evolve and adapt to the TME in liver cancer.

HSC-Derived CAFs Crosstalk with Tumor Cells

The development of mouse models, enabling genetic manipulation of HSC-derived CAFs and their secretome, has started to shed light on the functional heterogeneity of CAFs during liver cancer progression. For instance, iCCA and liver metastases are characterized by high tumor stiffness. Given that

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collagen type I is linked to stiffness, its role has been largely investigated in iCCA and liver metastasis. Despite reducing tumor stiffness and mechano-signaling pathways in both iCCA and PDAC liver metastases, surprisingly, *Col1a1* deletion in HSC-derived CAFs did not affect tumor growth in iCCA,[117] while it was tumor restrictive in PDAC metastases and primary tumors.[118,139] These results suggest that collagen type I exhibits distinct properties, either promoting tumor growth through mechanotransduction or acting as a mechanical barrier, which may be influenced by the tumor's origin, the pattern and localization of ECM accumulation, or other unknown factors. Interestingly, within the same myCAF signature, different mediators exhibit distinct properties. Hyaluronan synthase 2 (Has2), another myCAF mediator responsible for the production of hyaluronic acid (HA) in the liver, has been shown to promote tumor growth in both iCCA and liver metastasis despite not altering tumor stiffness,[117,118,140] which remains controversial. These findings show how different ECM components differentially impact tumor growth and stiffness, raising new questions about the tumor promoting or restricting functions of other CAF mediators in these tumors.

Although transcriptomic signatures have provided valuable insights into CAF heterogeneity, their functional and clinical relevance remains uncertain. However, recent studies suggest that integrating cross-species stroma, tumor, and immune microenvironment elements—starting from transcriptomic signatures—may help in stratifying patients, predicting response to therapy, and guiding preclinical studies.[138]

CAFs are also a substantial source of growth factors and cytokines that facilitate their crosstalk with other cells within the TME, including malignant cells. Indeed, HSC-derived CAFs are a major producer of HGF in iCCA and liver metastasis. Binding to its receptor c-MET, mostly expressed by tumor cells, HGF activates downstream kinase cascades and promotes tumor growth.[117,118] As mentioned earlier, HGF is also largely produced by qHSC and cyHSC and protects hepatocytes from liver injury.[13] This dual role of HGF highlights the complexity of targeting CAF-derived mediators in liver tumors. Although its pro-tumorigenic effects through c-MET activation in malignant cells make it an attractive therapeutic target, its hepatoprotective functions in the context of liver injury pose challenges for systemic inhibition.

Among the cytokines secreted by CAFs, IL-6 has been extensively studied over the years in liver carcinogenesis.[141] Recently, in iCCA, IL-6 was shown to be primarily secreted by vCAFs.[137] Unlike COL1A1 or HGF, there is a consensus on the tumor-promoting role of IL-6 in liver cancer[137,142] and metastasis.[143] By binding to the IL-6 receptor expressed by tumor cells, IL-6 activates downstream signals—mainly mediated by the STAT3 pathway—supporting tumor growth. Therefore, targeting this pathway seems a promising therapeutic strategy for liver cancer and metastases.[144,145]

These findings highlight how a single mediator can affect tumor progression differently, depending on its spatial location in the normal liver, PME, or TME, as well as its interactions with different cell types within the niche. These examples represent only a fraction of the numerous CAF mediators that directly affect tumor cells that have been described in the literature (for an extensive review, see Cantallops et al,[124] Ezhilarasan and Najimi,[146] and Montori et al[147]). However, many of these mediators have been investigated in *in vitro* systems or global knockout mice and would require further validation in more complex systems. Thanks to rapid technical advances and improved experimental models, future analysis integrating scRNA-seq and spatial transcriptomics, coupled with state-of-the-art animal models, will provide new knowledge on the functional role of CAFs in liver cancer.

Crosstalk Between CAFs and Other Stromal Components

As previously mentioned, the TME of iCCA is characterized by a dense fibrous and desmoplastic stroma, which is also accompanied by a complex tumor immune microenvironment. In iCCA CAFs contribute to extensive ECM remodeling, characterized by the breakdown of original components and the deposition of newly synthesized proteins, such as periostin and tenascin-C.[148] Periostin has been described to exert several tumor-promoting effects, by enhancing tumor cell proliferation and invasion through direct interaction with iCCA cells, and by recruiting tumor-associated macrophages (TAMs).[149] Similarly, tenascin-C has been shown to promote tumor proliferation and stimulate neighboring fibroblasts to release angiogenic factors.[150]

Another key CAF-produced ECM component, as previously mentioned, is COL1A1, which, in addition to its role in tumor stiffness and mechano-signaling,[136,151,152] has been also described to serve as a physical barrier to limit tumor growth in liver metastases[117] (►**Fig. 3**).

Moreover, desmoplasia in iCCA is accompanied by the presence of anti-inflammatory myeloid and macrophage populations, which together with CAFs mediate an immunosuppressive environment.[153] Like in other cancer types, immune infiltration in iCCA varies, with T and B cell infiltration correlating with better prognosis, while innate immune cells such as CD163+ immunosuppressive macrophages and neutrophils have been associated with poorer outcomes.[154–156] Moreover, high FOXP3+ Tregs/CD8+ T cells ratios have been linked to worse survival outcomes.[157] The iCCA TME is rich in immunosuppressive populations including MDSCs, tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), and Tregs[158–160] (►**Fig. 3**). Depletion of HSC-derived CAFs in animal models led to a decrease in Tregs,[117] highlighting the role of CAF in supporting Tregs, which is consistent across cancers (see above and Gao et al[161]), even if the mechanisms remain unclear in liver cancers. Therapeutic interventions targeting TAMs in cholangiocarcinoma have not been successful due to compensatory increases in MDSCs, driven by expression of CXCL12 by CAF.[162] Moreover, a CAF subset expressing high levels of periostin and ECM signatures has been linked to an immunosuppressive transcriptional program dependent on TGF-β signaling.[138] Thus, the interactions between CAFs, immune cells, and the ECM highlight the complexity of the TME and present multiple avenues for novel therapeutic interventions targeting immunosuppressive mechanisms in CCA.

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CAFs have also been shown to interact with endothelial cells in the TME of iCCA by secreting proangiogenic factors. Indeed, it has been shown that CAFs secrete VEGF-A and VEGF-C in response to PDGF-D, therefore affecting new vessel formation, an important step in the early metastasis processes in CCA.[163] Moreover, HSCs have been described to secrete several pro-angiogenic factors such as PDGF, fibroblast growth factor (FGF), TGF-β, EGF, ANGPT1, and ANGPT2, whose specific functional role in neo-angiogenesis remains to be explored further in iCCA.[124]

HSCs Targeting in Liver Cancer

Activated HSCs and HSC-derived CAFs are central players in the PME and TME of liver cancer. Although both are predominantly pro-tumorigenic, broad therapeutic strategies targeting them—such as TGF-β inhibition—have failed, and no Food and Drug Administration (FDA)-approved therapies currently exist to directly target aHSCs or CAFs. A major challenge has been the historically oversimplified view of HSCs as a homogeneous population. However, emerging insights into their diverse subtypes, states, functions, and anatomical niches emphasize the need for a deeper understanding of their specific roles, particularly in driving immunosuppressive mechanisms, for the development of more tailored and effective therapies (►**Table 3**).

Targeting HSC Activation and CAFs

Preserving homeostatic HSC functions while selectively targeting pathological activation is crucial for effective therapy. Although HSC activation drives fibrosis and immune evasion, these cells also support liver homeostasis. Rather than broadly depleting HSCs, targeting specific subsets—such as myofibroblastic HSCs remodeling ECM or senescent HSCs sustaining inflammation—may maximize therapeutic benefits while minimizing risks. Among the most explored approaches, TGF-β inhibition, the major pro-fibrogenic cytokine,[164] has long been considered a promising strategy for limiting HSC activation given its central role in fibrosis and tumor progression. However, clinical trials using TGF-β targeting antibodies (e.g., fresolimumab and metelimumab) have failed likely due to the pleiotropic nature of TGF-β, where its inhibition can have paradoxically promoted tumor progression.[165,166]

CAR T-cells have revolutionized the treatment of hematological malignancies and have recently emerged as a promising strategy for the TME targeting in solid tumors as well. However, CAR T-cell efficacy in solid tumors remains limited due to the immunosuppressive environment, impeding their infiltration and tumor cells heterogeneity.[167] Fibroblast activation protein (FAP)-targeting CAR T-cells, targeting a subpopulation of activated fibroblasts, have shown potential in preclinical cancer models by reducing ECM density, improving T-cell infiltration, and controlling tumor growth.[168–172] However, on-target off-tumor toxicity has been reported due to FAP expression in healthy tissues, including bone marrow stromal cells, leading to cachexia and anemia in mice.[173] Nevertheless, early-phase clinical trials have shown safety at sub-therapeutic doses in a phase I trial for pleural mesothelioma (NCT01722149)[174,175] and FAP-bispecific antibodies are currently under clinical evaluation.[176] Mesothelin (MSLN) CAR T-cells have also been explored in the context of solid tumors.[177] MSLN is highly expressed in multiple tumor types as well as in apCAFs in liver cancer.[178] In PDAC, apCAFs expressing MSLN have been shown to promote tumor invasion.[178] Preclinical MSLN-targeting CAR T-cell strategies have shown promising results in PDAC and ovarian cancer models,[179,180] but their utility in HCC and iCCA remains unknown.

To improve efficacy while minimizing toxicity, dual-targeting strategies are under development. For instance, nectin-4/FAP-CAR T-cells and MSLN-CAR T-cells have been engineered to secrete bispecific T-cell engagers against FAP and CD3, to simultaneously target FAP^+ CAFs and tumor cells.[181,182] In addition, CAR T-cells can release cytokines to elicit bystander effects on negative tumor cells, thereby broadening therapeutic impact in solid tumors.[181] Other emerging CAF markers like tumor endothelial marker 1 (TEM1) have emerged as promising targets. TEM1 have been shown to be strongly expressed by tumor-associated pericytes and perivascular CAFs and directed CAR T-cells led to CAF depletion, tumor vasculature disruption, and reduced metastasis in syngeneic models of breast tumor.[183–185]

Despite the promising potential of CAR T-cell therapies in liver cancer, we have to take into consideration that it comes accompanied by cytokine release syndrome (CRS) and compromised liver function, often observed in HCC patients, which remain a significant barrier for CAR T-cell therapy, particularly as CRS may exacerbate pre-existing hepatic dysfunction, potentially making this therapy less suitable for these individuals—a critical issue that warrants further investigation. Given these challenges, alternative approaches such as antibody-drug conjugates (ADCs) or nanoparticles may provide safer and more effective therapeutic options for patients with impaired hepatic function.

Targeting Senescent HSC-Derived CAFs

Another promising therapeutic approach is targeting senescent CAFs, independently from their cell of origin. The Bcl2 inhibitor Navitoclax (ABT-263) or Dasatinib/Quercetin combination (D + Q) has been used to target senescent cells including CAFs in iCCA and HCC in syngeneic experimental models.[49,125] However, although clinical trial is ongoing for D + Q combination (phase 1–2) for MASLD (NCT05506488), Navitoclax + sorafenib phase 1 did not show clinical benefit in HCC (NCT01364051). One main limitation of Navitoclax is its hematologic toxicity.[173] Recent studies in breast cancer and PDAC have expanded the scope of senolytic strategies, showing that targeting senescent CAFs can reduce immunosuppression and enhance immune infiltration, which underscores their potential in solid tumors, including liver cancer.[186,187] Novel approaches, such as uPAR-targeting CAR T-cells, have also shown promise in reducing fibrosis in MASH mouse model. Although this therapeutic approach is attractive, it might not target senescent cells only, same for the other agents, as uPAR is not only expressed on senescent cells but also on some subset of myeloid cells.

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Table 3 HSC targeting strategies in liver cancer

Therapeutic approach	Therapeutic target	Therapy	Effect	Reference
Targeting HSCs activation and CAFs	TGF-β signaling inhibition	Anti-TGFβ (e.g., fresolimumab and metelimumab)	Reduces HSC activation and myofibroblast differentiation but failed in clinical trials	Oh et al, 2024[203] Tacke et al, 2023[165] Henderson et al, 2020[166]
	FAP + CAF (including my CAF)	Bispecific antibodies and FAP-targeting, CAR T-cells	In preclinical models, reduce ECM, improve T-cell infiltration, control tumor growth	Sakemura et al, 2022[168] Liu et al, 2023[169] Das et al, 2023[170] Lo et al, 2015[171]
	MSLN + CAF (mes/CAF, apCAF and mesothelial cells)	MSLN-targeting CAR T-cells	Sustained antitumor effect, with a stronger antitumor response	Zhai et al, 2023[177] Huang et al, 2022[178] Amit et al, 2024[179] Schoutrop et al, 2021[180]
	Dual targeting: Nectin-4, FAP, MSLN	Nectin-4/FAP-CAR T-cells and MSLN-CAR T-cells	Simultaneous targeting of CAFs and tumor cells	Li et al, 2022[181] Wehrli et al, 2024[182]
	TEM1 expressing CAF	TEM1-directed CAR T-cells	CAF depletion, tumor vasculature disruption, and reduced metastasis in syngeneic models of breast tumor	Ash et al, 2024[183]
Targeting senescent HSC-derived CAFs	Senescent cells	Senolytics: Bcl2 inhibitor Navitoclax (ABT-263) or Dasatinib/Quercetin combination	Navitoclax + sorafenib phase 1 did not show clinical benefit in HCC (NCT01364051). In breast cancer and PDAC, targeting senescent CAFs can reduce immunosuppression and enhance immune infiltration.	Montori et al, 2022[147] Mertens et al, 2013[125]
	uPAR+ cells (upregulated in senescent cells)	uPAR-targeting CAR T-cells	Reduce fibrosis in MASH mouse model	Amor et al, 2020[48]
Targeting stiffness and ECM signaling	Collagen cross-linking activity of lysyl oxidase-like 2 (LOXL2)	Anti-LOXL2 antibodies (e.g., simtuzumab)	Improve T cell migration, reduces oxidative phosphorylation and tumor growth	Nicolas-Boluda et al, 2020[188] Lewinska et al, 2024[190]
	Collagen receptor DDR1	Dasatinib (approved by the US FDA) has activity against DDR1 but not specific	Reduce tumor proliferation in preclinical model of HCC	Filliol et al, 2022[13]
Targeting CAF-secreted mediators	IL-6/IL6R	Anti-IL-6, or IL-6Rantibodies (e.g., tocilizumab)	IL-6 neutralizing antibody blocks crosstalk between vascular CAF and tumor cells	Xu et al, 2021[204] Huang et al, 2022[144] Johnson et al, 2018[145]
	c-MET	c-MET inhibitor (e.g., cabozantinib)	Blocking HGF/cMet axis is expected to reduce tumor growth in iCCA, but showed limited efficacy and significant toxicity in CCA patients	Kakkar et al, 2007[194]

Abbreviations: CAF, cancer-associated fibroblast; CAR, chimeric antigen receptor; ECM, extracellular matrix; FAP, fibroblast activation protein; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; MASH, metabolic associated steatohepatitis; MSLN, mesothelin; PDAC, pancreatic ductal adenocarcinoma.

Targeting ECM and Matrix-Associated Signaling

Efforts to target the ECM and stiffness in liver cancer have also been explored, but remain challenging, particularly in contexts with dense ECM and highly crosslinked collagen. These strategies include the use of simtuzumab, an antibody designed to block the collagen cross-linking activity of lysyl oxidase-like 2 (LOXL2). Preclinical studies in iCCA demonstrate that tumor stiffness and extracellular matrix remodeling, driven by cancer-associated fibroblasts and LOX, play a critical role in tumor progression, immune evasion, and chemoresistance, with strategies such as photothermal therapy targeting tumor stiffness and LOX inhibition improving T cell migration, reducing oxidative phosphorylation, and hindering tumor growth.[188–190] However, clinical trials for advanced fibrosis or cirrhosis failed to demonstrate antifibrotic efficacy.[191]

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Discoidin domain receptor 1 (DDR1), a collagen-binding receptor tyrosine kinase, has emerged as another ECM-related target. DDR1 deletion had no effect on hepatocytes but promoted tumor reduction in mouse models.[13] In addition, DDR1 has been implicated in promoting tumor progression in breast and pancreatic cancers.[112,115] Although there is no DDR1-specific inhibitor, dasatinib (approved by the US FDA) shows activity against DDR1 and is currently in clinical trials in unresectable advanced HCC (NCT00459108).

A deeper understanding of the matrisome—the complete set of ECM components and associated proteins—will be critical for identifying novel therapeutic targets.[58,192] Given the ECM's dual role in both promoting immune exclusion and restraining tumor dissemination, future studies should explore combination therapies that selectively target immunosuppressive ECM features while preserving structural integrity to prevent metastasis.

Targeting CAF-Derived Cytokines and Growth Factors

CAF-secreted mediators play a pivotal role in shaping the TME, by influencing immune cell recruitment, ECM remodeling, and cancer cell survival. Targeting those interaction have been promising but some can complicate their therapeutic targeting due to their dual pro- and anti-tumoral role.

IL-6 is a targetable molecule that is under scrutiny in different clinical trials in HCC and CCA patients (NCT04338685), since its pro-tumorigenic role seems well-defined for both primary and metastatic liver cancers. Moreover, IL-6 receptor neutralization using tocilizumab may synergize with chemotherapy in dual therapies.[99] HGF, predominantly released by CAFs, plays a potent proliferative role in tumor cells through MET signaling, making it an attractive therapeutic target. However, due to its spatially dependent and opposing functions, its inhibition has been unsuccessful so far. For example, cabozantinib, a c-MET inhibitor, demonstrated limited efficacy and significant toxicity in CCA patients during a phase II clinical trial.[193] Similarly, rilotumumab, a human IgG2 monoclonal antibody against HGF, was discontinued in a phase III trial combining it with cisplatin and capecitabine for gastric cancer (NCT02137343) after increased mortality was observed.[194]

These advances highlight the potential of stroma-targeted strategies to overcome barriers in challenging malignancies like liver cancer. Other promising strategies include reprogramming CAFs into a quiescent state or remodeling the immune-excluded tumor microenvironment to foster a more immune-permissive and inflamed state, enhancing responsiveness to ICB therapy. By enabling T-cell infiltration and mitigating immune exhaustion, these approaches could aim to transform liver cancer's immunosuppressive landscape into one that is more conducive to effective treatment. However, although ECM degradation or manipulation may facilitate T-cell infiltration and enhance immunotherapy efficacy, it is important to acknowledge that HSCs and CAFs also establish a protective, cocoon-like structure around tumors. This barrier may not only support tumor growth but also constrain dissemination, raising critical considerations for therapeutic targeting. Therefore, an optimal therapeutic strategy may involve a combination approach: selectively targeting pathological CAF/HSC subsets while preserving structural integrity to prevent metastatic escape. Combining these approaches with tyrosine kinase inhibitors or ICB could help counterbalance the risks of disrupting ECM homeostasis while enhancing anti-tumor immunity. Although still speculative, these concepts underscore the potential for precision approaches to significantly improve outcomes in liver cancer.

Concluding Remarks

Despite the increasing interest in HSCs and CAFs in the PME and TME of liver tumors, more studies are needed to carefully define the functional role of specific HSC mediators involved in liver fibrosis and tumorigenesis. Indeed, although tremendous effort has resulted in the characterization of transcriptomic heterogeneity during activation, little is known yet about the functional meaning of such heterogeneity both in the PME and TME of liver cancer and metastasis. Given the dynamic role of HSCs, the ECM, and the CAFs, future therapeutic strategies will likely require a multi-pronged approach, ideally combining ECM remodeling or CAF reprogramming, with current therapies such as chemotherapy or ICB, to ensure precise targeting while maximizing the efficacy without promoting tumor dissemination. The advent of innovative spatial technologies, including transcriptomic and multiplex imaging, may contribute to better characterize HSCs, HSC-derived CAFs, and their mediators, not only improving our understanding of fibrotic niches within the PME and TME but also enabling patient stratification based on stromal composition. These techniques will help in exploring specific niches in the fibrotic liver and may be useful in validating new therapeutic targets.

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Conflict of Interest
None declared.

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